10/525,365

```
=> d his

     (FILE 'HOME' ENTERED AT 13:52:46 ON 20 MAR 2007)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 13:54:04 ON 20 MAR 2007
L1        119294 S FLUORESCENT (4W)PROTEIN?
L2            32 S TRACHYPHYLLIA (W)GEOFFROYI
L3            23 S L1 AND L2
L4            11 DUP REM L3 (12 DUPLICATES REMOVED)
                 E MIYAWAKI A/AU
L5           510 S E3
                 E ANDO R/AU
L6           588 S E3
                 E KARASAWA S/AU
L7            35 S E12
                 E MIZUNO H/AU
L8          2076 S E3
L9          3149 S L5 OR L6 OR L7 OR L8
L10          225 S L1 AND L9
L11           12 S L2 AND L10
L12            6 DUP REM L11 (6 DUPLICATES REMOVED)
```

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LOGINID:ssspta1652mxm

PASSWORD:
TERMINAL (ENTER 1, 2, 3, OR ?):2

* * * * * * * * * * Welcome to STN International * * * * * * * * * *

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 NEWS  1          Web Page URLs for STN Seminar Schedule - N. America
 NEWS  2          "Ask CAS" for self-help around the clock
 NEWS  3  DEC 18  CA/CAplus pre-1967 chemical substance index entries enhanced
                  with preparation role
 NEWS  4  DEC 18  CA/CAplus patent kind codes updated
 NEWS  5  DEC 18  MARPAT to CA/CAplus accession number crossover limit increased
                  to 50,000
 NEWS  6  DEC 18  MEDLINE updated in preparation for 2007 reload
 NEWS  7  DEC 27  CA/CAplus enhanced with more pre-1907 records
 NEWS  8  JAN 08  CHEMLIST enhanced with New Zealand Inventory of Chemicals
 NEWS  9  JAN 16  CA/CAplus Company Name Thesaurus enhanced and reloaded
 NEWS 10  JAN 16  IPC version 2007.01 thesaurus available on STN
 NEWS 11  JAN 16  WPIDS/WPINDEX/WPIX enhanced with IPC 8 reclassification data
 NEWS 12  JAN 22  CA/CAplus updated with revised CAS roles
 NEWS 13  JAN 22  CA/CAplus enhanced with patent applications from India
 NEWS 14  JAN 29  PHAR reloaded with new search and display fields
 NEWS 15  JAN 29  CAS Registry Number crossover limit increased to 300,000 in
                  multiple databases
 NEWS 16  FEB 15  PATDPASPC enhanced with Drug Approval numbers
 NEWS 17  FEB 15  RUSSIAPAT enhanced with pre-1994 records
 NEWS 18  FEB 23  KOREAPAT enhanced with IPC 8 features and functionality
 NEWS 19  FEB 26  MEDLINE reloaded with enhancements
 NEWS 20  FEB 26  EMBASE enhanced with Clinical Trial Number field
 NEWS 21  FEB 26  TOXCENTER enhanced with reloaded MEDLINE
 NEWS 22  FEB 26  IFICDB/IFIPAT/IFIUDB reloaded with enhancements
 NEWS 23  FEB 26  CAS Registry Number crossover limit increased from 10,000
                  to 300,000 in multiple databases
 NEWS 24  MAR 15  WPIDS/WPIX enhanced with new FRAGHITSTR display format
 NEWS 25  MAR 16  CASREACT coverage extended
 NEWS 26  MAR 20  MARPAT now updated daily

 NEWS EXPRESS  NOVEMBER 10 CURRENT WINDOWS VERSION IS V8.01c, CURRENT
               MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),
               AND CURRENT DISCOVER FILE IS DATED 25 SEPTEMBER 2006.

 NEWS HOURS    STN Operating Hours Plus Help Desk Availability
 NEWS LOGIN    Welcome Banner and News Items
 NEWS IPC8     For general information regarding STN implementation of IPC 8
 NEWS X25      X.25 communication option no longer available
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Enter NEWS followed by the item number or name to see news on that specific topic.

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* * * * * * * * * * * * * * * * STN  Columbus  * * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 13:52:46 ON 20 MAR 2007

=> s fluroscent (4w)protein?
THIS COMMAND NOT AVAILABLE IN THE CURRENT FILE
Some commands only work in certain files.  For example, the EXPAND
command can only be used to look at the index in a file which has an
index.  Enter "HELP COMMANDS" at an arrow prompt (=>) for a list of
commands which can be used in this file.

=> s fluorescent (4w)protein?
THIS COMMAND NOT AVAILABLE IN THE CURRENT FILE
Some commands only work in certain files.  For example, the EXPAND
command can only be used to look at the index in a file which has an
index.  Enter "HELP COMMANDS" at an arrow prompt (=>) for a list of
commands which can be used in this file.

=> file emdline embase biosis biotechds scisearch hcaplus ntis lifesci
'EMDLINE' IS NOT A VALID FILE NAME
Enter "HELP FILE NAMES" at an arrow prompt (=>) for a list of files
that are available.  If you have requested multiple files, you can
specify a corrected file name or you can enter "IGNORE" to continue
accessing the remaining file names entered.
ENTER A FILE NAME OR (IGNORE):medline
COST IN U.S. DOLLARS                            SINCE FILE      TOTAL
                                                   ENTRY    SESSION
FULL ESTIMATED COST                                 0.42       0.42

FILE 'MEDLINE' ENTERED AT 13:54:04 ON 20 MAR 2007

FILE 'EMBASE' ENTERED AT 13:54:04 ON 20 MAR 2007
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FILE 'BIOSIS' ENTERED AT 13:54:04 ON 20 MAR 2007
Copyright (c) 2007 The Thomson Corporation

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COPYRIGHT (C) 2007 THE THOMSON CORPORATION

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FILE 'HCAPLUS' ENTERED AT 13:54:04 ON 20 MAR 2007
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FILE 'NTIS' ENTERED AT 13:54:04 ON 20 MAR 2007
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FILE 'LIFESCI' ENTERED AT 13:54:04 ON 20 MAR 2007
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=> s fluorescent (4w)protein?
L1      119294 FLUORESCENT (4W) PROTEIN?

=> s trachyphyllia (w)geoffroyi
L2          32 TRACHYPHYLLIA (W) GEOFFROYI
```

```
=> s l1 and l2
L3          23 L1 AND L2

=> dup rem l3
PROCESSING COMPLETED FOR L3
L4          11 DUP REM L3 (12 DUPLICATES REMOVED)

=> d 1-11 ibib ab

L4   ANSWER 1 OF 11  HCAPLUS  COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER:        2006:1252707  HCAPLUS
DOCUMENT NUMBER:         146:23029
TITLE:                   Optical microscopy with phototransformable optical
                         labels
INVENTOR(S):             Hess, Harald F.; Betzig, Robert E.
PATENT ASSIGNEE(S):      USA
SOURCE:                  PCT Int. Appl., 75pp.
                         CODEN: PIXXD2
DOCUMENT TYPE:           Patent
LANGUAGE:                English
FAMILY ACC. NUM. COUNT:  1
PATENT INFORMATION:

     PATENT NO.           KIND   DATE        APPLICATION NO.         DATE
     ---------------      ----   --------    --------------------    --------
     WO 2006127692         A2    20061130    WO 2006-US19887         20060523
         W:  AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH,
             CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD,
             GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR,
             KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX,
             MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE,
             SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC,
             VN, YU, ZA, ZM, ZW
         RW: AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE,
             IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ,
             CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG, BW, GH,
             GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY,
             KG, KZ, MD, RU, TJ, TM
PRIORITY APPLN. INFO.:                       US 2005-683337P     P  20050523
                                             US 2006-780968P     P  20060310
AB   First activation radiation is provided to a sample that includes
     phototransformable optical labels ("PTOLs") to activate a first subset of
     the PTOLs in the sample.  First excitation radiation is provided to the
     first subset of PTOLs in the sample to excite at least some of the
     activated PTOLs, and radiation emitted from activated and excited PTOLs
     within the first subset of PTOLs is detecting with imaging optics.  The
     first activation radiation is controlled such that the mean volume per
     activated PTOLs in the first subset is greater than or approx. equal to a
     diffraction-limited resolution volume ("DLRV") of the imaging optics.

L4   ANSWER 2 OF 11     MEDLINE on STN                    DUPLICATE 1
ACCESSION NUMBER:   2006712529     MEDLINE
DOCUMENT NUMBER:    PubMed ID: 17092037
TITLE:              Competition between energy and proton transfer in ultrafast
                    excited-state dynamics of an oligomeric fluorescent
                    protein red Kaede.
AUTHOR:             Hosoi Haruko; Mizuno Hideaki; Miyawaki Atsushi; Tahara
                    Tahei
CORPORATE SOURCE:   Molecular Spectroscopy Laboratory, RIKEN (The Institute of
                    Physical and Chemical Research), Wako, Saitama 351-0198,
                    Japan.
SOURCE:             The journal of physical chemistry. B, Condensed matter,
                    materials, surfaces, interfaces & biophysical, (2006 Nov
                    16) Vol. 110, No. 45, pp. 22853-60.
```

Journal code: 101157530. ISSN: 1520-6106.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200701
ENTRY DATE: Entered STN: 12 Dec 2006
Last Updated on STN: 27 Jan 2007
Entered Medline: 26 Jan 2007

AB We investigated femtosecond and picosecond time-resolved fluorescence dynamics of a tetrameric fluorescent protein Kaede with a red chromophore (red Kaede) to examine a relationship between the excited-state dynamics and a quaternary structure of the fluorescent protein. Red Kaede was obtained by photoconversion from green Kaede that was cloned from a stony coral Trachyphyllia geoffroyi. In common with other typical fluorescent proteins, a chromophore of red Kaede has two protonation states, the neutral and the anionic forms in equilibrium. Time-resolved fluorescence measurements clarified that excitation of the neutral form gives the anionic excited state with a time constant of 13 ps at pH 7.5. This conversion process was attributed to fluorescence resonance energy transfer (FRET) from the photoexcited neutral form to the ground-state anionic form that is located in an adjacent subunit in the tetramer. The time-resolved fluorescence data measured at different pH revealed that excited-state proton transfer (ESPT) also occurs with a time constant of 300 ps and hence that the FRET and ESPT take place simultaneously in the fluorescent protein as competing processes. The ESPT rate in red Kaede was significantly slower than the rate in Aequorea GFP, which highly likely arises from the different hydrogen bond network around the chromophore.

L4 ANSWER 3 OF 11 MEDLINE on STN
ACCESSION NUMBER: 2006452697 MEDLINE
DOCUMENT NUMBER: PubMed ID: 16753144
TITLE: Dynamic behavior of individual cells in developing organotypic brain slices revealed by the photoconvertable protein Kaede.
AUTHOR: Mutoh T; Miyata T; Kashiwagi S; Miyawaki A; Ogawa M
CORPORATE SOURCE: Laboratory for Cell Culture Development, Advanced Technology Development Center, Brain Science Institute, Riken Saitama, Japan.. tmuto@brain.riken.jp
SOURCE: Experimental neurology, (2006 Aug) Vol. 200, No. 2, pp. 430-7. Electronic Publication: 2006-06-06.
Journal code: 0370712. ISSN: 0014-4886.
PUB. COUNTRY: United States
DOCUMENT TYPE: (COMPARATIVE STUDY)
(IN VITRO)
Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200609
ENTRY DATE: Entered STN: 1 Aug 2006
Last Updated on STN: 22 Sep 2006
Entered Medline: 21 Sep 2006

AB In recent years, advances in optical imaging methods have facilitated the visualization of events in the developing cortex. In particular, the introduction of DNA encoding fluorescent protein into cells of the embryonic brain allows the visualization of progenitor cells; slice preparations of the cortex then allow the monitoring of the behavior of transfected cells in the context of the living cerebral wall by time-lapse microscopy. Such approaches have provided substantial information about the patterns of neuronal migration. However, as these

techniques label large numbers of cells in the ventricular zone (VZ), it is difficult to follow individual cell shape changes or cell behaviors within the VZ, where neuron production and initial migration take place. Here, we report a unique method using the photoconvertable fluorescent protein Kaede, which emits green fluorescence and shifts to emitting red fluorescence upon radiation with UV. Using this method, we were able to follow the behavior of a particular pair of daughter cells among neighboring Kaede-positive cells in the SVZ of mouse brain slices. The spindle shape progenitor divided into two multipolar-shaped daughter cells. The cell-cell borders of daughter cells were clearly visualized, and easily describe the position and distance between two or more cells. The photoconvertable property of Kaede offers a powerful cell marking tool to identify the precise morphology and migratory behaviors of individual cells within living cortical slices.

L4 ANSWER 4 OF 11 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
ACCESSION NUMBER: 2007:30318 BIOSIS
DOCUMENT NUMBER: PREV200700030032
TITLE: The 1.7 angstrom crystal structure of Dronpa: A photoswitchable green fluorescent protein

AUTHOR(S): Wilmann, Pascal G.; Turcic, Kristina; Battad, Jion M.; Wilce, Matthew C. J.; Devenish, Rodney J.; Prescott, Mark [Reprint Author]; Rossjohn, Jamie
CORPORATE SOURCE: Monash Univ, Prot Crystallog Unit, Clayton, Vic 3800, Australia
Mark.Prescott@med.monash.edu.au; Jamie.Rossjohn@med.monash.edu.au
SOURCE: Journal of Molecular Biology, (NOV 24 2006) Vol. 364, No. 2, pp. 213-224.
CODEN: JMOBAK. ISSN: 0022-2836.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 27 Dec 2006
Last Updated on STN: 27 Dec 2006

AB The green fluorescent protein (GFP), its variants, and the closely related GFP-like proteins possess a wide variety of spectral properties that are of widespread interest as biological tools. One desirable spectral property termed photoswitching, involves the light-induced alteration of the optical properties of certain GFP members. Although the structural basis of both reversible and irreversible photoswitching events have begun to be unraveled, the mechanisms resulting in reversible photoswitching are less clear. A novel GFP-like protein, Dronpa, was identified to have remarkable light-induced photoswitching properties, maintaining an almost perfect reversible photochromic behavior with a high fluorescence to dark state ratio. We have crystallized and subsequently determined to 1.7 angstrom resolution the crystal structure of the fluorescent state of Dronpa. The chromophore was observed to be in its anionic form, adopting a cis co-planar conformation. Comparative structural analysis of non-photoactivatable and photoactivatable GFPs, together with site-directed mutagenesis of a position (Cys62) within the Dronpa chromophore, has provided a basis for understanding Dronpa photoactivation. Specifically, we propose a model of reversible photoactivation whereby irradiation with light leads to subtle conformational changes within and around the environment of the chromophore that promotes proton transfer along an intricate polar network.

L4 ANSWER 5 OF 11 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
ACCESSION NUMBER: 2006:569901 BIOSIS
DOCUMENT NUMBER: PREV200600582693
TITLE: Cloning and characterization of novel fluorescent proteins from Anthozoan animals and their

applications to cell biological research.
AUTHOR(S): Karasawa, Satoshi; Miyawaki, Atsushi
SOURCE: Zoological Science (Tokyo), (DEC 2005) Vol. 22, No. 12, pp. 1417-1418.
Meeting Info.: 76th Annual Meeting of the Zoological-Society-of-Japan. Tsukuba, JAPAN. October 06 -08, 2005. Zool Soc Japan.
CODEN: ZOSCEX. ISSN: 0289-0003.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 1 Nov 2006
Last Updated on STN: 1 Nov 2006

L4 ANSWER 6 OF 11 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 2005110192 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15731765
TITLE: Semi-rational engineering of a coral fluorescent protein into an efficient highlighter.
AUTHOR: Tsutsui Hidekazu; Karasawa Satoshi; Shimizu Hideaki; Nukina Nobuyuki; Miyawaki Atsushi
CORPORATE SOURCE: Laboratory for Cell Function Dynamics, Brain Science Institute, RIKEN, 2-1 Hirosawa, Wako-city, Saitama 351-0198, Japan.
SOURCE: EMBO reports, (2005 Mar) Vol. 6, No. 3, pp. 233-8.
Journal code: 100963049. ISSN: 1469-221X.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200508
ENTRY DATE: Entered STN: 3 Mar 2005
Last Updated on STN: 9 Aug 2005
Entered Medline: 8 Aug 2005

AB Kaede is a natural photoconvertible fluorescent protein found in the coral Trachyphyllia geoffroyi. It contains a tripeptide, His 62-Tyr 63-Gly 64, which acts as a green chromophore that is photoconvertible to red following (ultra-) violet irradiation. Here, we report the molecular cloning and crystal structure determination of a new fluorescent protein, KikG, from the coral Favia favus, and its in vitro evolution conferring green-to-red photoconvertibility. Substitution of the His 62-Tyr 63-Gly 64 sequence into the native protein provided only negligible photoconversion. On the basis of the crystal structure, semi-rational mutagenesis of the amino acids surrounding the chromophore was performed, leading to the generation of an efficient highlighter, KikGR. Within mammalian cells, KikGR is more efficiently photoconverted and is several-fold brighter in both the green and red states than Kaede. In addition, KikGR was successfully photoconverted using two-photon excitation microscopy at 760 nm, ensuring optical cell labelling with better spatial discrimination in thick and highly scattering tissues.

L4 ANSWER 7 OF 11 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
ACCESSION NUMBER: 2005:483889 BIOSIS
DOCUMENT NUMBER: PREV200510287888
TITLE: Applications of fluorescent protein, kaede, to Arabidopsis.
AUTHOR(S): Yamamoto, Junko [Reprint Author]; Arimura, Shin-ichi; Tsutsumi, Nobuhiro
CORPORATE SOURCE: Univ Tokyo, Grad Sch Agr and Sci, Tokyo, Japan
SOURCE: Plant and Cell Physiology, (2005) Vol. 46, No. Suppl. S, pp. S148.
Meeting Info.: 46th Annual Meeting of the

Japanese-Society-of-Plant-Physiologists. Niigata, JAPAN. March 24 -26, 2005. Japanese Soc Plant Physiologists. CODEN: PCPHA5. ISSN: 0032-0781.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 16 Nov 2005
Last Updated on STN: 16 Nov 2005

L4 ANSWER 8 OF 11 BIOTECHDS COPYRIGHT 2007 THE THOMSON CORP. on STN
DUPLICATE 3
ACCESSION NUMBER: 2004-14562 BIOTECHDS
TITLE: New modified green fluorescent protein, useful as a reporter in expression studies; modified fusion protein prepared by directed evolution useful as a reporter gene protein
AUTHOR: WALDO G S
PATENT ASSIGNEE: WALDO G S
PATENT INFO: US 2004078148 22 Apr 2004
APPLICATION INFO: US 2003-423688 24 Apr 2003
PRIORITY INFO: US 2003-423688 24 Apr 2003; US 2002-132067 24 Apr 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-340059 [31]
AB DERWENT ABSTRACT:
NOVELTY - A green fluorescent protein that comprises at least 80% identity to a fully defined sequence of 56 amino acids (SEQ ID NO:1); (b) at least one amino acid substitution selected from e.g., a substitution at position 30 that is an arginine or a conservative variant of arginine; and (c) measurable fluorescence activity.

DETAILED DESCRIPTION - A green fluorescent protein that comprises at least 80% identity to a fully defined sequence of 56 amino acids (SEQ ID NO:1); (b) at least one amino acid substitution selected from the group consisting of a substitution at position 30 that is an arginine or a conservative variant of arginine; a substitution at position 39 that is an asparagine or a conservative variant of asparagine; a substitution at position 105 that is a threonine or a conservative variant of threonine; a substitution at position 171 that is a valine; and a substitution at position 206 that is a valine; where the positions are determined in alignment for maximal correspondence with SEQ ID NO:1; and (c) measurable fluorescence activity. INDEPENDENT CLAIMS are also included for the following: (1) directed-evolution (M1) of generating enhanced folding variant of target polypeptide, comprising mutating a polynucleotide encoding polypeptide of interest to generate a library of mutated polynucleotides, linking mutated polynucleotides to polynucleotide encoding folding interference domain to form fusion protein (FP) constructs, expressing FP, and selecting FP that display optimal folding activity in relation to FP comprising wild-type polypeptide and folding interference domain, thus identifying polypeptide with enhanced folding activity.(2) an enhanced folding variant (I) of a fluorescent protein generated by (M1); (3) an enhanced folding variant (II) of a chromophoric protein generated by (M1); (4) enhancing (M2) folding of a polypeptide comprising multiple domains, involves joining a first domain of the polypeptide to a poorly folding domain, to form a fusion protein, mutating the first domain, detecting an increase in the amount of activity generated by a first mutated fusion protein in comparison to a fusion protein comprising a wild-type first domain and the poorly folding polypeptide domain, thus identifying a first domain with enhanced folding, joining a second domain of the polypeptide to the first mutated fusion protein to form a second fusion protein, mutating the second domain, and detecting an increase in the amount of activity generated by a second mutated fusion protein in comparison to a fusion protein comprising the wild-type second domain and the first mutated fusion protein, thus identifying a target polypeptide

with multiple domains that have enhanced folding; (5) an isolated nucleic acid (IV) encoding a green fluorescent protein that has at least 80% identity to (S1), at least one amino acid substitution as described above, and measurable fluorescent activity; (6) an expression vector (V) comprising (IV); and (7) a host cell comprising (V).

BIOTECHNOLOGY - Preferred Methods: (M1) further involves carrying out one or more subsequent rounds of directed evolution on a fusion protein construct encoding an initially selected fusion protein that display optimal folding activity, and selecting fusion proteins from it with optimal folding activity relative to the folding activity of the initially selected fusion protein, thus identifying polypeptides with further enhanced folding activity. The folding activity is determined by measuring folding kinetics or by measuring resistance to denaturation by urea. The folding activity of the fusion protein is determined by measuring a biological activity of the target polypeptide. The target polypeptide is a fluorescent protein and the biological activity is fluorescence. The target polypeptide is a chromophoric protein and the biological activity is color. The fluorescent protein is chosen from Aequorea victoria green fluorescent protein and a fluorescent protein having a structure with a root mean square deviation of less than 5Angstrom from the 11- stranded beta-barrel component of the A.victoria Green fluorescent protein structure MMDB Id: 5742. Preferred Variant: In (I), the fluorescent protein is A.victoria green fluorescent protein , red fluorescent protein from a Discosoma species, Anthosoan fluorescent protein, Trachyphyllia geoffroyi, or Anemonia sulcata. In (II), the fluorescent protein has a fully defined amino acid sequence of 182 amino acids (S2), or 168 amino acids (S3). The target polypeptide is a reporter polypeptide. The fusion protein further comprises a reporter molecule that has the reporter activity. The reporter activity is chosen from fluorescent signal or antibiotic resistance. The poorly folding domain is a ferritin domain. The fusion protein comprises a target polypeptide linked to the poorly folding domain by a linker. The reporter molecule is linked to the fusion protein. The folding interference domain is inserted into permissive sites of the target polypeptide. The target polypeptide is green fluorescent protein that has at least 80% identity when aligned for maximum correspondence to (S2) or to a fully defined sequence of 180 amino acids (S4), and has fluorescent activity. Preferred Protein: (III) further comprises a phenylalanine substitution at position 145. The amino acid substitution is chosen from arginine substitution at position 30, an asparagine substitution position 39, threonine substitution at position 105, valine substitution at position 171, and a valine substitution at position 206. The substitution is arginine at position 30, asparagine at position 39, threonine at position 105, phenylalanine at position 145, valine at position 171, or valine at position 206. The green fluorescent protein comprises any two, three, four, or five substitutions as described in (III). The five substitutions are an arginine at position 30, asparagine at position 39, threonine at position 105, valine at position 171 and valine at position 206. The green fluorescent protein further comprises a sixth substitution that is a phenylalanine at position 145. (III) further comprises a mutation chosen from Phe99Ser, Met153Thr and Val163Ala. (III) is cyclized. Preferred Nucleic Acid: (IV) encodes a green fluorescent protein having at least one amino acid substitution chosen from arginine substitution at position 30, asparagine substitution at position 39, threonine substitution at position 105, phenylalanine substitution at position 145, valine substitution at position 171, and a valine substitution at position 206.

USE - (I) and (II) are useful as reporter proteins to express the report level of a protein. (M1) is useful for directed-evolution of

generating enhanced folding variant of target polypeptide (claimed). (M1) is useful for improving folding and solubility of a target protein.

ADVANTAGE - (I) provides new and more stable scaffolds for the creation of new GFP variants.

EXAMPLE - To test the effect of the superfolder mutations, 6 single-point mutants of cycle-3 redshift were engineered by PCR. Each mutant incorporated one of the 6 mutations found the superholder green fluorescent protein (GFP) variant. These were cloned into a pET vector as C-terminal fusions with poorly-folded bullfrog redcell ferritin. Rapid protein-folding assay using green fluorescent protein was carried out. Overnight cultures in Luria-Bertani (LB) media containing kanamycin (35 g/ml) were diluted 100-fold and grown for 2 hours at 37degreesC. Proteins were expressed for 4 hours by adding isopropyl-D-thiogalactopyranoside (IPTG) to 1 mM in 3 ml cultures of LB media at either 37degreesC or 27degreesC in Escherichia coli BL21(DE3) as C-terminal fusions with poorly-folded bull frog red cell H-subunit ferritin. Cycle-3 redshift and superfolder were cloned and expressed similarly as controls, both with and without the N-terminal ferritin. The fluorescence (488 nm ex/520 nm em) and absorbance (600 nm) were measured for each culture using a BioTek FL-600 plate reader. (46 pages)

L4 ANSWER 9 OF 11 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 2004:183021 HCAPLUS
DOCUMENT NUMBER: 140:231205
TITLE: Chromogenic and fluorescent proteins from coral and use as optical indicator based on the UV-induced green-to-red photoconversion
INVENTOR(S): Miyawaki, Atsushi; Ando, Ryoko; Karasawa, Satoshi; Mizuno, Hideaki
PATENT ASSIGNEE(S): Riken Corp., Japan; Medical & Biological Laboratories Co., Ltd.
SOURCE: PCT Int. Appl., 118 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2004018671	A1	20040304	WO 2003-JP10628	20030822
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
CA 2499755	A1	20040304	CA 2003-2499755	20030822
AU 2003262274	A1	20040311	AU 2003-262274	20030822
EP 1548107	A1	20050629	EP 2003-792798	20030822
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK				
US 2006160990	A1	20060720	US 2005-525365	20051012

PRIORITY APPLN. INFO.:		
JP 2002-243337	A	20020823
JP 2002-243338	A	20020823
JP 2002-274266	A	20020920
JP 2002-280118	A	20020926
WO 2003-JP10628	W	20030822

AB The invention provides a chromogenic proteins originating in Anthopleura

inornata having definite chromogenic properties; and fluorescent proteins originating in Trachyphyllia geoffroyi and Scolymia vitiensis having definite fluorescent properties, and use as fluorescent indicator. Those proteins can be expressed as fusion protein with the protein of interest to study localization or dynamics in vivo using FRET. The authors cloned cDNAs encoding chromogenic proteins from Anthopleura inornata, which they named Be-G and Be-R. Sequences for the cDNAs and putative proteins encoded were determined and the proteins were recombinantly expressed in E. coli, and optical properties were studied. The authors also cloned a gene encoding a fluorescent protein from a stony coral, Trachyphyllia geoffroyi, which emits green, yellow, and red light. The protein, named Kaede, includes a tripeptide, His-Tyr-Gly, that acts as a green chromophore that can be converted to red. The red fluorescence is comparable in intensity to the green and is stable under usual aerobic conditions. They found that the green-red conversion is highly sensitive to irradiation with UV or violet light (350-400 nm), which excites the protonated form of the chromophore. The excitation lights used to elicit red and green fluorescence do not induce photoconversion. Under a conventional epifluorescence microscope, Kaede protein expressed in HeLa cells turned red in a graded fashion in response to UV illumination; maximal illumination resulted in a 2,000-fold increase in the ratio of red-to-green signal. These color-changing properties provide a simple and powerful technique for regional optical marking. Another fluorescent protein was cloned from Scolymia vitiensis and named Momiji. Various mutants were produced by engineering, including dimer and monomer.

REFERENCE COUNT: 9 THERE ARE 9 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 10 OF 11 HCAPLUS COPYRIGHT 2007 ACS on STN

ACCESSION NUMBER: 2003:886090 HCAPLUS
DOCUMENT NUMBER: 140:107188
TITLE: Photo-induced peptide cleavage in the green-to-red conversion of a fluorescent protein
AUTHOR(S): Mizuno, Hideaki; Mal, Tapas Kumar; Tong, Kit I.; Ando, Ryoko; Furuta, Toshiaki; Ikura, Mitsuhiko; Miyawaki, Atsushi
CORPORATE SOURCE: Laboratory for Cell Function Dynamics, Advanced Technology Development Group, Brain Science Institute, The Institute of Physical and Chemical Science (RIKEN), Wako-city, 351-0198, Japan
SOURCE: Molecular Cell (2003), 12(4), 1051-1058
CODEN: MOCEFL; ISSN: 1097-2765
PUBLISHER: Cell Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Green fluorescent protein from the jellyfish (Aequorea GFP) and GFP-like proteins from coral species encode light-absorbing chromophores within their protein sequences. A coral fluorescent protein, Kaede, contains a tripeptide, His62-Tyr63-Gly64, which acts as a green chromophore that is photoconverted to red. Here, the authors present the structural basis for the green-to-red photoconversion. As in Aequorea GFP, a chromophore, 4-(p-hydroxybenzylidene)-5-imidazolinone, derived from the tripeptide mediates green fluorescence in Kaede. UV irradiation causes an unconventional cleavage within Kaede protein between the amide nitrogen and the α carbon (Cα) at His62 via a formal β-elimination reaction, which requires the whole, intact protein for its catalysis. The subsequent formation of a double bond between His62-Cα and -Cβ extends the π-conjugation to the imidazole ring of His62, creating a new red-emitting chromophore, 2-[(1E)-2-(5-imidazolyl)ethenyl]-4-(p-hydroxybenzylidene)-5-imidazolinone. The present study not only reveals diversity in the chemical structure of fluorescent proteins but also adds a new dimension to

posttranslational modification mechanisms.
REFERENCE COUNT: 23 THERE ARE 23 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 11 OF 11 MEDLINE on STN DUPLICATE 4
ACCESSION NUMBER: 2002616608 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12271129
TITLE: An optical marker based on the UV-induced green-to-red photoconversion of a fluorescent protein

AUTHOR: Ando Ryoko; Hama Hiroshi; Yamamoto-Hino Miki; Mizuno Hideaki; Miyawaki Atsushi
CORPORATE SOURCE: Laboratory for Cell Function and Dynamics, Advanced Technology Development Center, Brain Science Institute, The Institute of Physical and Chemical Research (RIKEN), 2-1 Hirosawa, Wako-city, Saitama 351-0198, Japan.
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (2002 Oct 1) Vol. 99, No. 20, pp. 12651-6. Electronic Publication: 2002-09-23.
Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AB085641
ENTRY MONTH: 200211
ENTRY DATE: Entered STN: 11 Oct 2002
Last Updated on STN: 5 Jan 2003
Entered Medline: 13 Nov 2002

AB We have cloned a gene encoding a fluorescent protein from a stony coral, Trachyphyllia geoffroyi, which emits green, yellow, and red light. The protein, named Kaede, includes a tripeptide, His-Tyr-Gly, that acts as a green chromophore that can be converted to red. The red fluorescence is comparable in intensity to the green and is stable under usual aerobic conditions. We found that the green-red conversion is highly sensitive to irradiation with UV or violet light (350-400 nm), which excites the protonated form of the chromophore. The excitation lights used to elicit red and green fluorescence do not induce photoconversion. Under a conventional epifluorescence microscope, Kaede protein expressed in HeLa cells turned red in a graded fashion in response to UV illumination; maximal illumination resulted in a 2,000-fold increase in the ratio of red-to-green signal. These color-changing properties provide a simple and powerful technique for regional optical marking. A focused UV pulse creates an instantaneous plane source of red Kaede within the cytosol. The red spot spreads rapidly throughout the cytosol, indicating its free diffusibility in the compartment. The extensive diffusion allows us to delineate a single neuron in a dense culture, where processes originating from many different somata are present. Illumination of a focused UV pulse onto the soma of a Kaede-expressing neuron resulted in filling of all processes with red fluorescence, allowing visualization of contact sites between the red and green neurons of interest.

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E2            2     MIYAWAKI/AU
E3          510 --> MIYAWAKI A/AU
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E5            1     MIYAWAKI AKIHIKO/AU
E6            1     MIYAWAKI AKIMITSU/AU
E7            7     MIYAWAKI AKINOBU/AU
E8           23     MIYAWAKI AKIRA/AU
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E10           1      MIYAWAKI AKITSUGU/AU
E11          13      MIYAWAKI AKIYOSHI/AU
E12           3      MIYAWAKI AKIYUKI/AU

=> s e3
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'AUY' IS NOT A VALID EXPAND FIELD CODE FOR FILE 'HCAPLUS'
'AUY' IS NOT A VALID EXPAND FIELD CODE FOR FILE 'EMBASE'
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file.  To see a list of valid EXPAND field codes, enter HELP
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E3          588 --> ANDO R/AU
E4           12      ANDO R A/AU
E5            1      ANDO R C/AU
E6            9      ANDO R D/AU
E7           11      ANDO R E/AU
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E10           2      ANDO R R/AU
E11          28      ANDO REIKO/AU
E12           6      ANDO RICHARD A/AU

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L6          588 "ANDO R"/AU

=> e karasawa s/au
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E2            7      KARASAWA RYOU/AU
E3          206 --> KARASAWA S/AU
E4            1      KARASAWA S I/AU
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E10           2      KARASAWA SAKAE/AU
E11          43      KARASAWA SATORU/AU
E12          35      KARASAWA SATOSHI/AU

=> s e12
L7           35 "KARASAWA SATOSHI"/AU

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E2            1      MIZUNO GYOU/AU
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E8            9      MIZUNO HANA/AU
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E11           1      MIZUNO HARUKUNI/AU
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L8         2076 "MIZUNO H"/AU

=> d his

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     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 13:54:04 ON 20 MAR 2007
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L2            32 S TRACHYPHYLLIA (W)GEOFFROYI
L3            23 S L1 AND L2
L4            11 DUP REM L3 (12 DUPLICATES REMOVED)
                 E MIYAWAKI A/AU
L5           510 S E3
                 E ANDO R/AU
L6           588 S E3
                 E KARASAWA S/AU
L7            35 S E12
                 E MIZUNO H/AU
L8          2076 S E3

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L9         3149 L5 OR L6 OR L7 OR L8

=> s l1 and l9
L10         225 L1 AND L9

=> s l2 and l10
L11          12 L2 AND L10

=> dup rem l11
PROCESSING COMPLETED FOR L11
L12             6 DUP REM L11 (6 DUPLICATES REMOVED)

=> d 1-6 ibib ab

L12  ANSWER 1 OF 6  SCISEARCH  COPYRIGHT (c) 2007 The Thomson Corporation  on
     STN
ACCESSION NUMBER:    2006:1132171  SCISEARCH
THE GENUINE ARTICLE: 103RZ
TITLE:               Competition between energy and proton transfer in
                     ultrafast excited-state dynamics of an oligomeric
                     fluorescent protein red kaede
AUTHOR:              Hosoi H; Mizuno H; Miyawaki A; Tahara
                     T (Reprint)
CORPORATE SOURCE:    RIKEN Inst Phys & Chem Res, Mol Spectr Lab, 2-1 Hirosawa,
                     Wako, Saitama 3510198, Japan (Reprint); RIKEN Inst Phys &
                     Chem Res, Mol Spectr Lab, Wako, Saitama 3510198, Japan;
                     RIKEN, Brain Sci Inst, Adv Technol Dev Grp, Lab Cell Funct
                     Dynam, Wako, Saitama 3510198, Japan
                     tahei@riken.jp
COUNTRY OF AUTHOR:   Japan
SOURCE:              JOURNAL OF PHYSICAL CHEMISTRY B, (16 NOV 2006) Vol. 110,
                     No. 45, pp. 22853-22860.
                     ISSN: 1520-6106.
PUBLISHER:           AMER CHEMICAL SOC, 1155 16TH ST, NW, WASHINGTON, DC 20036
                     USA.
DOCUMENT TYPE:       Article; Journal
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LANGUAGE: English
REFERENCE COUNT: 29
ENTRY DATE: Entered STN: 30 Nov 2006
Last Updated on STN: 30 Nov 2006
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB We investigated femtosecond and picosecond time-resolved fluorescence dynamics of a tetrameric fluorescent protein Kaede with a red chromophore (red Kaede) to examine a relationship between the excited-state dynamics and a quaternary structure of the fluorescent protein. Red Kaede was obtained by photoconversion from green Kaede that was cloned from a stony coral Trachyphyllia geoffroyi. In common with other typical fluorescent proteins, a chromophore of red Kaede has two protonation states, the neutral and the anionic forms in equilibrium. Time-resolved fluorescence measurements clarified that excitation of the neutral form gives the anionic excited state with a time constant of 13 ps at pH 7.5. This conversion process was attributed to fluorescence resonance energy transfer (FRET) from the photoexcited neutral form to the ground-state anionic form that is located in an adjacent subunit in the tetramer. The time-resolved fluorescence data measured at different pH revealed that excited-state proton transfer (ESPT) also occurs with a time constant of 300 ps and hence that the FRET and ESPT take place simultaneously in the fluorescent protein as competing processes. The ESPT rate in red Kaede was significantly slower than the rate in Aequorea GFP, which highly likely arises from the different hydrogen bond network around the chromophore.

L12 ANSWER 2 OF 6 MEDLINE on STN
ACCESSION NUMBER: 2006452697 MEDLINE
DOCUMENT NUMBER: PubMed ID: 16753144
TITLE: Dynamic behavior of individual cells in developing organotypic brain slices revealed by the photoconvertable protein Kaede.
AUTHOR: Mutoh T; Miyata T; Kashiwagi S; Miyawaki A; Ogawa M
CORPORATE SOURCE: Laboratory for Cell Culture Development, Advanced Technology Development Center, Brain Science Institute, Riken Saitama, Japan.. tmuto@brain.riken.jp
SOURCE: Experimental neurology, (2006 Aug) Vol. 200, No. 2, pp. 430-7. Electronic Publication: 2006-06-06.
Journal code: 0370712. ISSN: 0014-4886.
PUB. COUNTRY: United States
DOCUMENT TYPE: (COMPARATIVE STUDY)
(IN VITRO)
Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200609
ENTRY DATE: Entered STN: 1 Aug 2006
Last Updated on STN: 22 Sep 2006
Entered Medline: 21 Sep 2006

AB In recent years, advances in optical imaging methods have facilitated the visualization of events in the developing cortex. In particular, the introduction of DNA encoding fluorescent protein into cells of the embryonic brain allows the visualization of progenitor cells; slice preparations of the cortex then allow the monitoring of the behavior of transfected cells in the context of the living cerebral wall by time-lapse microscopy. Such approaches have provided substantial information about the patterns of neuronal migration. However, as these techniques label large numbers of cells in the ventricular zone (VZ), it is difficult to follow individual cell shape changes or cell behaviors within the VZ, where neuron production and initial migration take place. Here, we report a unique method using the photoconvertable

fluorescent protein Kaede, which emits green fluorescence and shifts to emitting red fluorescence upon radiation with UV. Using this method, we were able to follow the behavior of a particular pair of daughter cells among neighboring Kaede-positive cells in the SVZ of mouse brain slices. The spindle shape progenitor divided into two multipolar-shaped daughter cells. The cell-cell borders of daughter cells were clearly visualized, and easily describe the position and distance between two or more cells. The photoconvertable property of Kaede offers a powerful cell marking tool to identify the precise morphology and migratory behaviors of individual cells within living cortical slices.

L12 ANSWER 3 OF 6 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
ACCESSION NUMBER: 2006:569901 BIOSIS
DOCUMENT NUMBER: PREV200600582693
TITLE: Cloning and characterization of novel fluorescent proteins from Anthozoan animals and their applications to cell biological research.
AUTHOR(S): Karasawa, Satoshi; Miyawaki, Atsushi
SOURCE: Zoological Science (Tokyo), (DEC 2005) Vol. 22, No. 12, pp. 1417-1418.
Meeting Info.: 76th Annual Meeting of the Zoological-Society-of-Japan. Tsukuba, JAPAN. October 06 -08, 2005. Zool Soc Japan.
CODEN: ZOSCEX. ISSN: 0289-0003.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 1 Nov 2006
Last Updated on STN: 1 Nov 2006

L12 ANSWER 4 OF 6 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 2005110192 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15731765
TITLE: Semi-rational engineering of a coral fluorescent protein into an efficient highlighter.
AUTHOR: Tsutsui Hidekazu; Karasawa Satoshi; Shimizu Hideaki; Nukina Nobuyuki; Miyawaki Atsushi
CORPORATE SOURCE: Laboratory for Cell Function Dynamics, Brain Science Institute, RIKEN, 2-1 Hirosawa, Wako-city, Saitama 351-0198, Japan.
SOURCE: EMBO reports, (2005 Mar) Vol. 6, No. 3, pp. 233-8.
Journal code: 100963049. ISSN: 1469-221X.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200508
ENTRY DATE: Entered STN: 3 Mar 2005
Last Updated on STN: 9 Aug 2005
Entered Medline: 8 Aug 2005

AB Kaede is a natural photoconvertible fluorescent protein found in the coral Trachyphyllia geoffroyi. It contains a tripeptide, His 62-Tyr 63-Gly 64, which acts as a green chromophore that is photoconvertible to red following (ultra-) violet irradiation. Here, we report the molecular cloning and crystal structure determination of a new fluorescent protein, KikG, from the coral Favia favus, and its in vitro evolution conferring green-to-red photoconvertibility. Substitution of the His 62-Tyr 63-Gly 64 sequence into the native protein provided only negligible photoconversion. On the basis of the crystal structure, semi-rational mutagenesis of the amino acids surrounding the chromophore was performed, leading to the generation of an efficient highlighter, KikGR. Within mammalian cells, KikGR is more

efficiently photoconverted and is several-fold brighter in both the green and red states than Kaede. In addition, KikGR was successfully photoconverted using two-photon excitation microscopy at 760 nm, ensuring optical cell labelling with better spatial discrimination in thick and highly scattering tissues.

L12 ANSWER 5 OF 6 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 2004:183021 HCAPLUS
DOCUMENT NUMBER: 140:231205
TITLE: Chromogenic and fluorescent proteins from coral and use as optical indicator based on the UV-induced green-to-red photoconversion
INVENTOR(S): Miyawaki, Atsushi; Ando, Ryoko; Karasawa, Satoshi; Mizuno, Hideaki
PATENT ASSIGNEE(S): Riken Corp., Japan; Medical & Biological Laboratories Co., Ltd.
SOURCE: PCT Int. Appl., 118 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2004018671	A1	20040304	WO 2003-JP10628	20030822
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
CA 2499755	A1	20040304	CA 2003-2499755	20030822
AU 2003262274	A1	20040311	AU 2003-262274	20030822
EP 1548107	A1	20050629	EP 2003-792798	20030822
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK				
US 2006160990	A1	20060720	US 2005-525365	20051012

PRIORITY APPLN. INFO.:		
JP 2002-243337	A	20020823
JP 2002-243338	A	20020823
JP 2002-274266	A	20020920
JP 2002-280118	A	20020926
WO 2003-JP10628	W	20030822

AB The invention provides a chromogenic proteins originating in Anthopleura inornata having definite chromogenic properties; and fluorescent proteins originating in Trachyphyllia geoffroyi and Scolymia vitiensis having definite fluorescent properties, and use as fluorescent indicator. Those proteins can be expressed as fusion protein with the protein of interest to study localization or dynamics in vivo using FRET. The authors cloned cDNAs encoding chromogenic proteins from Anthopleura inornata, which they named Be-G and Be-R. Sequences for the cDNAs and putative proteins encoded were determined and the proteins were recombinantly expressed in E. coli, and optical properties were studied. The authors also cloned a gene encoding a fluorescent protein from a stony coral, Trachyphyllia geoffroyi, which emits green, yellow, and red light. The protein, named Kaede, includes a tripeptide, His-Tyr-Gly, that acts as a green chromophore that can be converted to red. The red fluorescence is comparable in intensity to the green and is stable under usual aerobic conditions. They found that the green-red conversion is highly sensitive

to irradiation with UV or violet light (350-400 nm), which excites the protonated form of the chromophore. The excitation lights used to elicit red and green fluorescence do not induce photoconversion. Under a conventional epifluorescence microscope, Kaede protein expressed in HeLa cells turned red in a graded fashion in response to UV illumination; maximal illumination resulted in a 2,000-fold increase in the ratio of red-to-green signal. These color-changing properties provide a simple and powerful technique for regional optical marking. Another fluorescent protein was cloned from Scolymia vitiensis and named Momiji. Various mutants were produced by engineering, including dimer and monomer.

REFERENCE COUNT: 9 THERE ARE 9 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 6 OF 6 EMBASE COPYRIGHT (c) 2007 Elsevier B.V. All rights reserved on STN DUPLICATE 2

ACCESSION NUMBER: 2002360292 EMBASE

TITLE: An optical marker based on the UV-induced green-to-red photoconversion of a fluorescent protein

AUTHOR: Ando R.; Hama H.; Yamamoto-Hino M.; Mizuno H.; Miyawaki A.

CORPORATE SOURCE: A. Miyawaki, Laboratory for Cell Function, Adv. Technology Development Center, Brain Science Institute, 2-1 Hirosawa, Wako-city, Saitama 351-0198, Japan. matsushi@brain.riken.go.jp

SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1 Oct 2002) Vol. 99, No. 20, pp. 12651-12656. .
Refs: 19
ISSN: 0027-8424 CODEN: PNASA6

COUNTRY: United States

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 008 Neurology and Neurosurgery
029 Clinical Biochemistry

LANGUAGE: English

SUMMARY LANGUAGE: English

ENTRY DATE: Entered STN: 24 Oct 2002
Last Updated on STN: 24 Oct 2002

AB We have cloned a gene encoding a fluorescent protein from a stony coral, Trachyphyllia geoffroyi, which emits green, yellow, and red light. The protein, named Kaede, includes a tripeptide, His-Tyr-Gly, that acts as a green chromophore that can be converted to red. The red fluorescence is comparable in intensity to the green and is stable under usual aerobic conditions. We found that the green-red conversion is highly sensitive to irradiation with UV or violet light (350-400 nm), which excites the protonated form of the chromophore. The excitation lights used to elicit red and green fluorescence do not induce photoconversion. Under a conventional epifluorescence microscope, Kaede protein expressed in HeLa cells turned red in a graded fashion in response to UV illumination; maximal illumination resulted in a 2,000-fold increase in the ratio of red-to-green signal. These color-changing properties provide a simple and powerful technique for regional optical marking. A focused UV pulse creates an instantaneous plane source of red Kaede within the cytosol. The red spot spreads rapidly throughout the cytosol, indicating its free diffusibility in the compartment. The extensive diffusion allows us to delineate a single neuron in a dense culture, where processes originating from many different somata are present. Illumination of a focused UV pulse onto the soma of a Kaede-expressing neuron resulted in filling of all processes with red fluorescence, allowing visualization of contact sites between the red and green neurons of interest.

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     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 13:54:04 ON 20 MAR 2007
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L2            32 S TRACHYPHYLLIA (W)GEOFFROYI
L3            23 S L1 AND L2
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                 E MIYAWAKI A/AU
L5           510 S E3
                 E ANDO R/AU
L6           588 S E3
                 E KARASAWA S/AU
L7            35 S E12
                 E MIZUNO H/AU
L8          2076 S E3
L9          3149 S L5 OR L6 OR L7 OR L8
L10          225 S L1 AND L9
L11           12 S L2 AND L10
L12            6 DUP REM L11 (6 DUPLICATES REMOVED)
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	Issue Date	Pages	Document ID	Title
1	20070308	98	US 20070053934 A1	Polyvalent multimeric composition containing active polypeptides, pharmaceutical compositions and methods of using the same
2	20070104	168	US 20070003936 A1	Methods for the detection of colorectal cancer
3	20061116	104	US 20060258562 A1	Methods and pharmaceutical compositions for healing wounds
4	20061116	83	US 20060257887 A1	Protein -protein interaction detection system using fluorescent protein microdomains
5	20060831	98	US 20060194282 A1	Fluorescent protein variants and methods for making same
6	20060727	22	US 20060165597 A1	Methods for cancer imaging
7	20060720	53	US 20060160990 A1	Fluorescent protein and chromoprotein
8	20060720	40	US 20060160169 A1	Cell microarray for profiling of cellular phenotypes and gene function
9	20060720	34	US 20060159620 A1	Human kininogen D3 domain polypeptide as an anti-angiogenic and anti-tumor agent
10	20060706	79	US 20060148104 A1	Detection of ion channel or receptor activity
11	20060706	66	US 20060147413 A1	Photochemical activation of surfaces for attaching biomaterial

	Issue Date	Pages	Document ID	Title
12	20060629	30	US 20060142386 A1	Bis(thio-hydrazide amides) for increasing Hsp70 expression
13	20060525	115	US 20060110827 A1	Red and near infrared flourescent phyotochrome
14	20060511	61	US 20060099638 A1	Modified carbocyanine dyes and their conjugates
15	20060413	78	US 20060078893 A1	Compartmentalised combinatorial chemistry by microfluidic control
16	20060413	79	US 20060078888 A1	In vitro evolution in microfluidic systems
17	20060406	187	US 20060073474 A1	Methods and compositions for detecting the activation state of multiple proteins in single cells
18	20060119	37	US 20060015961 A1	Methods and compositions for producing germ cells from peripheral blood derived germline stem cells
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